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Original article

Effects of gemfibrozil or simvastatin on apolipoprotein-B-containing lipoproteins, apolipoprotein-CIII and lipoprotein(a) in familial combined hyperlipidaemia¹

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Abstract

Background: Familial combined hyperlipidaemia (FCH), characterized by elevated very-low-density lipoprotein (VLDL) and/or low-density lipoprotein (LDL), is associated with an increased prevalence of premature cardiovascular disease. Therefore, lipid-lowering is frequently indicated. **Methods:** We evaluated in a parallel, double-blind randomized fashion the effect of gemfibrozil (1200 mg/day) ($n = 40$) or simvastatin (20 mg/day) ($n = 41$) on lipids, apolipoprotein-B (apo-B)-containing lipoproteins, apo-CIII and lipoprotein(a) [Lp(a)], in 81 well-defined FCH patients. **Results:** While both drugs lowered plasma cholesterol and triglyceride levels, gemfibrozil lowered plasma triglycerides more effectively by reduction of triglycerides in VLDL and LDL, whereas simvastatin was more effective in its reduction of total plasma cholesterol by exclusively decreasing LDL cholesterol. High-density lipoprotein (HDL) increased to an equal extent on both therapies. Total serum apo-B levels were reduced with both drugs; however, gemfibrozil decreased apo-B only in VLDL + IDL, whereas simvastatin decreased apo-B in both VLDL + IDL and LDL. In keeping with a more effective reduction of VLDL particles, a more pronounced reduction of apo-CIII also was observed after gemfibrozil, which correlated with the reduction in plasma triglycerides. Baseline concentrations of Lp(a) showed a wide range in both treatment groups. Median Lp(a) levels increased after simvastatin, but were not affected by gemfibrozil. **Conclusion:** Both therapies exhibited their specific effects, although none of the drugs alone completely normalized the lipid profiles of these patients with FCH. Therefore, the choice of treatment should be based on the most elevated lipoprotein fraction, and in some cases a combination of the two drugs may be indicated.

Keywords: Lipids; Therapeutics; Clinical trial

1. Introduction

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Familial combined hyperlipidaemia (FCH) is the most common of the hereditary lipid disorders, with

an estimated prevalence of 0.5–1.0% in the general population and characterized by a strong predisposition to premature cardiovascular diseases in patients and first-degree relatives [1]. Affected individuals have elevated very-low-density lipoprotein (VLDL) concentrations, low-density lipoprotein (LDL) concentrations, or both. Furthermore, a low high-density lipoprotein (HDL) cholesterol concentration, an elevated apolipoprotein-B (apo-B) concentration [2], and an increased prevalence of atherogenic small dense LDL subfractions are observed [3–5]. Because of the absence of a specific clinical or metabolic marker for the disorder, and because of characteristic variability in the presenting lipid phenotype, family investigation is pivotal to establish the diagnosis FCH in a single patient [2]. FCH seems to be a metabolically and genetically heterogeneous lipid disorder [6] which, in general, is explained by an overproduction of VLDL particles [7,8]. Furthermore, an impaired hydrolytic capacity of lipoprotein lipase (LpL) itself [9], or elevated plasma levels of a LpL inhibitor apo-CIII, possibly linked to genetic polymorphisms in the AI-CIII-AIV gene cluster on chromosome 11 [10,11], may be related to the FCH phenotypes.

In general, elevated concentrations of lipoprotein(a) [Lp(a)] are related to an increased risk for cardiovascular disease, which is independent of the cardiovascular risk associated with elevated lipid levels [12]. Although FCH is not associated with elevated Lp(a) levels [13], Lp(a) concentrations correlate positively with LpL-mediated metabolism of triglyceride-rich lipoproteins [14]. Reduction of the elevated pool of triglyceride-rich lipoproteins, as seen in FCH, may influence plasma Lp(a) levels [15].

Because of the high incidence of cardiovascular diseases in these patients, lipid-lowering treatment should be initiated. If diet and lifestyle changes have insufficient effect, drug therapy may be indicated. For this purpose, the HMG-CoA reductase inhibitor, simvastatin, is highly effective in patients with primary hypercholesterolaemia [16], and fibrates like gemfibrozil with a primary triglyceride-lowering effect [17] are useful.

To date, few comparative data are available on the different effects of these agents in FCH. In the present study we describe the baseline levels of lipids, apo-B-containing lipoproteins, apo-CIII and Lp(a) in well-defined patients with FCH, and com-

pare the results of treatment with either gemfibrozil or simvastatin on these parameters.

2. Materials and methods

2.1. Subjects

In total, 81 FCH probands from 3 centres participated in this study. A subgroup of this population has been studied regarding LDL subfraction profiles and LDL oxidizability [5]. In the present study the effects of treatment with either gemfibrozil or simvastatin on serum lipids, lipoproteins, apo-CIII and Lp(a) in all 81 FCH probands are presented.

At the end of the screening period, all FCH patients met the following criteria: (1) a total serum cholesterol > 6.5 mmol/l and triglyceride level between 2.3 and 5.6 mmol/l, without hypolipidaemic drugs and on a standard lipid-lowering diet (30 energy % fat, P/S ratio of 2:1 and a cholesterol intake < 300 mg/day); (2) at least 1 first-degree relative with significant hypercholesterolaemia, hypertriglyceridaemia or both; (3) a positive family history of premature coronary heart disease; (4) total apo-B levels above 1200 mg/l. Furthermore, all patients were > 30 years old and patients with secondary causes for dyslipidaemia or with apolipoprotein phenotype E 2/2 were excluded. The study protocol was approved by the local medical ethical committees, and all patients participated after informed consent was obtained.

2.2. Experimental design

This study was a double-blind trial with a double dummy design, divided into a screening period (weeks –8 to –5), a baseline placebo period (weeks –4 to day 0) and an active treatment period (day 0 to week 12). In the screening period, patients were taken off all hypolipidaemic drugs, receiving a standard lipid-lowering diet. When total plasma cholesterol concentration exceeded 6.5 mmol/l, and plasma triglyceride concentration remained between 2.3 and 5.6 mmol/l, the patients entered the baseline placebo period of the study. During this period, each patient received 2 bottles, one containing placebo matching gemfibrozil and one containing placebo matching

simvastatin. During the active treatment period, patients were randomly assigned to receive either simvastatin 20 mg/day together with a placebo matching gemfibrozil ($n = 41$) or gemfibrozil 1200 mg/day together with a placebo matching simvastatin ($n = 40$). In the present study we compared concentrations of total plasma cholesterol and plasma triglycerides obtained at the end of the entry period, at the end of the placebo period and at two time points (6 and 12 weeks) during the period of active treatment. Furthermore, analyses of lipoproteins, apoproteins and Lp(a) at the end of the placebo-controlled period were compared with the same data at the end of the active treatment period.

2.3. Plasma

Blood samples were obtained after an overnight fast and collected in ethylenediaminetetraacetic acid (EDTA) (1 mg/ml)-containing vacutainers. Plasma was isolated immediately and a saccharose solution (600 mg/ml H₂O) was added to prevent denaturation of VLDL and LDL during freezing; samples were stored at -80°C for 4 to 15 weeks. The analysis of apo-CIII and Lp(a) was performed in the Lipid Research Laboratory, University Hospital of Utrecht; apo-B was determined in the Lipid Research Laboratory, University Hospital of Amsterdam; all other lipid determinations were performed in the Lipid Research Laboratory, University Hospital of Nijmegen.

2.4. Analytical methods

Very-low-density lipoproteins (VLDL) and intermediate-density lipoproteins (IDL) were isolated together by ultracentrifugation at density ≤ 1.019 g/ml for 16 h at 40 000 rpm in a fixed-angle rotor (TFT 45.6 rotor, Kontron, Zürich), in a Beckman L7-55 ultracentrifuge (Beckman, Palo Alto, USA) [18]. After removal of VLDL + IDL, we measured cholesterol and triglycerides in the remaining plasma and in total plasma. HDL was determined from whole plasma by the polyethylene glycol method [19]. All cholesterol and triglyceride measurements were determined by enzymatic methods (Boehringer Mannheim, Mannheim, Germany, cat. no. 237574 and Sera Pak, Tournai, Belgium, cat. no. 6669,

respectively) with a centrifugal analyzer (Multistat III; Instrumentation Laboratory, Lexington, MA). VLDL + IDL cholesterol and triglyceride were calculated by subtraction. Apo-B and apo-AI concentrations in total plasma and apo-B concentrations in the fraction that remained after VLDL + IDL removal were determined by nephelometry [20]. VLDL + IDL-apo-B was calculated by subtraction. Apo-CIII was determined quantitatively by radial immunodiffusion using plates and apo-CIII standards, according to the manufacturer's instructions (Daichi Pure Chemicals, Ltd, Japan). The diameter of the precipitation ring was measured by an investigator unaware of the specimen's identity. Serial samples from each subject were measured in the same assay. Lp(a) concentrations were determined by measuring the apoprotein(a) moiety in a commercially available solid-phase two-site immunoradiometric assay (IRMA) using 2 different specific anti-apoprotein(a) monoclonal antibodies (Pharmacia, Uppsala, Sweden).

2.5. Statistics

Results are expressed as mean \pm SD and as median for Lp(a). Statistical analysis of alterations within one group of treatment was performed with Wilcoxon's Signed Rank test. Differences in terms of percentage between the two groups of treatment were analyzed with the Mann-Whitney test. A two-tailed probability value of less than 0.05 was considered to be significant. The statistical analysis were performed with procedures available in the SPSS PC + (Statistical Package for the Social Sciences) software package Version 4.0 (SPSS Inc. Chicago, IL, USA)

3. Results

3.1. Participants

After the screening period (4 weeks), participants who exhibited a IIb phenotype (total plasma cholesterol concentration ≥ 6.5 mmol/l and plasma triglyceride concentration ≥ 2.3 mmol/l) were allowed to enter the placebo period. Due to variability in presenting lipid phenotype during the placebo

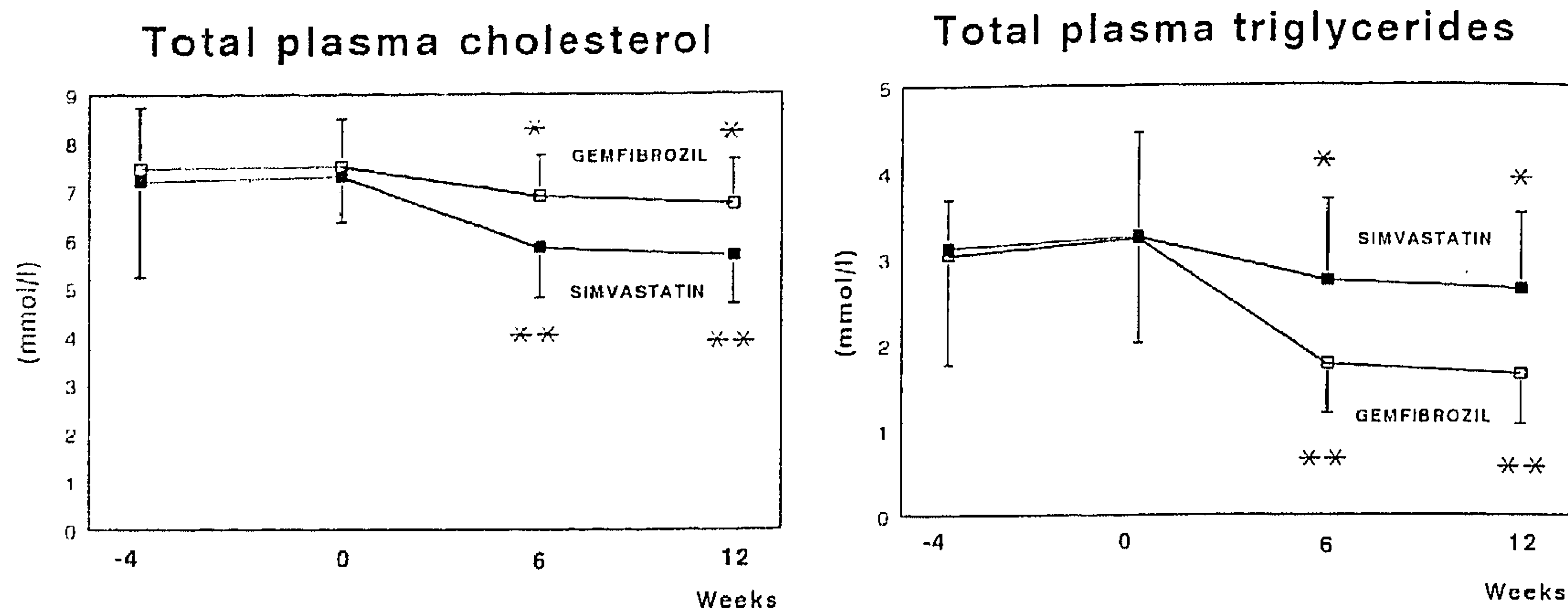


Fig. 1. Change in plasma concentrations (mmol/l) of total cholesterol (TC) and triglycerides (TG) during the placebo period (week -4 to day 0), and at two time-points (week 6 and week 12) during the period of active treatment with either gemfibrozil (\square) ($n = 40$) or simvastatin (\blacksquare) ($n = 41$) of patients with familial combined hyperlipidaemia. * $P < 0.001$, ** $P < 0.0001$, compared with the baseline level (week 0).

period (4 weeks), 55 patients exhibited a phenotype IIb, 12 patients exhibited a phenotype IIa (total plasma cholesterol concentration ≥ 6.5 and plasma triglyceride concentrations < 2.3 mmol/l), and 14 patients exhibited a phenotype IV (total plasma cholesterol concentration < 6.5 mmol/l and plasma triglyceride concentration ≥ 2.3 mmol/l, respectively) at the end of the baseline placebo period. Description of the two treatment groups, the body

mass index (BMI), and the blood pressure are presented in Table 1.

3.2. Effect of treatment on lipid and lipoprotein concentrations

In Fig. 1 the changes in the mean total plasma cholesterol and plasma triglyceride concentrations during the placebo period and the period of treatment

Table 2

Lipid and lipoprotein concentrations at the end of the placebo period (= before) and after treatment (= after) with either gemfibrozil ($n = 40$) or simvastatin ($n = 41$) of patients with familial combined hyperlipidaemia

	Drug	Before	After	Delta (%)	P
TC	G	7.50 \pm 0.98	6.73 \pm 0.91	-9.5 \pm 13.2	0.0001
	S	7.30 \pm 0.96	5.67 \pm 1.00	-22.4 \pm 9.2 ^{aa}	< 0.0001
TG	G	3.22 \pm 1.22	1.64 \pm 0.59	-45.5 \pm 19.7	< 0.0001
	S	3.20 \pm 1.20	2.63 \pm 0.88	-14.6 \pm 27.8 ^{aa}	0.0003
HDL-C	G	0.91 \pm 0.20	1.07 \pm 0.26	17.4 \pm 17.6	< 0.0001
	S	0.84 \pm 0.19	0.95 \pm 0.21	13.9 \pm 13.1	< 0.0001
VLDL + IDL-C	G	2.43 \pm 0.84	1.15 \pm 0.84	-50.6 \pm 20.6	< 0.0001
	S	2.60 \pm 1.02	1.56 \pm 0.62	-37.6 \pm 20.1 ^{aa}	< 0.0001
VLDL + IDL-TG	G	2.61 \pm 1.18	1.43 \pm 1.15	-44.7 \pm 28.0	< 0.0001
	S	2.59 \pm 1.04	2.08 \pm 0.79	-14.1 \pm 32.6 ^{aa}	0.0008
LDL-C	G	4.08 \pm 0.99	4.29 \pm 1.26	8.3 \pm 41.9	n.s.
	S	3.87 \pm 0.98	3.16 \pm 0.87	-16.3 \pm 21.0 ^{aa}	< 0.0001
LDL-TG	G	0.67 \pm 0.20	0.49 \pm 0.13	-22.9 \pm 19.9	< 0.0001
	S	0.60 \pm 0.12	0.53 \pm 0.12	-10.9 \pm 16.8 ^a	0.0003

Values are presented in mmol/l as mean \pm SD. TC = total plasma cholesterol; TG = plasma triglycerides; VLDL = very-low-density lipoproteins; IDL = intermediate-density lipoproteins; HDL = high-density lipoproteins; LDL = low-density lipoproteins; G = gemfibrozil, S = simvastatin; n.s., not significant. ^a $P < 0.001$, ^{aa} $P < 0.0001$, S versus G.

Table 1
Age, gender, blood pressure and body mass index of 81 patients with familial combined hyperlipidaemia

	Gemfibrozil	Simvastatin
<i>n</i>	40	41
Age (years)	53.4 ± 9.4	50.4 ± 10.8
Gender M/F	26/14	32/9
Systolic blood pressure (mmHg)	131 ± 18	127 ± 13
Diastolic blood pressure (mmHg)	84 ± 9	81 ± 8
Body mass index (kg/m ²)	27.2 ± 3.0	26.6 ± 2.7

Values are presented as mean ± SD.

are presented. At entry and at the end of the placebo period there were no significant differences in mean lipid and lipoprotein concentrations between the two groups, and as shown in Fig. 1, mean lipid levels were unaffected during the placebo period. Lipoprotein levels at the end of the baseline period and after therapy are summarized in Table 2. During the treatment period, the BMI appeared to be constant in both groups. Gemfibrozil affected total triglyceride levels in plasma as well as in the VLDL + IDL and LDL fraction significantly more than simvastatin, whereas simvastatin induced the largest reduction in total plasma cholesterol with an exclusive reduction of LDL-cholesterol. Gemfibrozil tended to increase LDL-cholesterol (+8.3%, not significant). In both groups, the reduction of cholesterol and triglycerides in the VLDL + IDL fraction was responsible for the major decrease on total plasma cholesterol and triglycerides. With both drugs, HDL-cholesterol concentrations increased significantly to an equal extent.

3.3. Effect of treatment on apo-B-containing lipoproteins

Alterations in the apo-B concentrations in total plasma, the VLDL + IDL fraction and the LDL fraction are presented in Fig. 2. Simvastatin and gemfibrozil reduced total plasma apo-B (−25.6 and −13.7%, respectively, but simvastatin was more effective if measured in percentage of change, $P = 0.02$). Simvastatin reduced apo-B in both the VLDL + IDL and the LDL fraction (−35.4 and 16.7%, respectively). Gemfibrozil, on the contrary, reduced apo-B only in the VLDL + IDL fraction (−42.4%; difference simvastatin vs. gemfibrozil in terms of percentage change, $P < 0.0001$), without affecting the LDL fraction (+5.5%, not significant; difference simvastatin vs. gemfibrozil in terms of percentage $P < 0.0001$).

3.4. Effect of treatment on apo-AI and apo-CIII

Changes in apo-AI and apo-CIII are summarized in Table 3. The increase in HDL-cholesterol on both treatments was not accompanied by an increase of apo-AI in both groups. Apo-CIII decreased significantly with both therapies, but more markedly after gemfibrozil. In general, the absolute decrease in Apo-CIII concentrations coincided with the decrease in VLDL + IDL particles, as measured by apo-B concentrations in this fraction. There was a significant correlation between the changes in apo-CIII and the change in plasma triglycerides after gemfibrozil

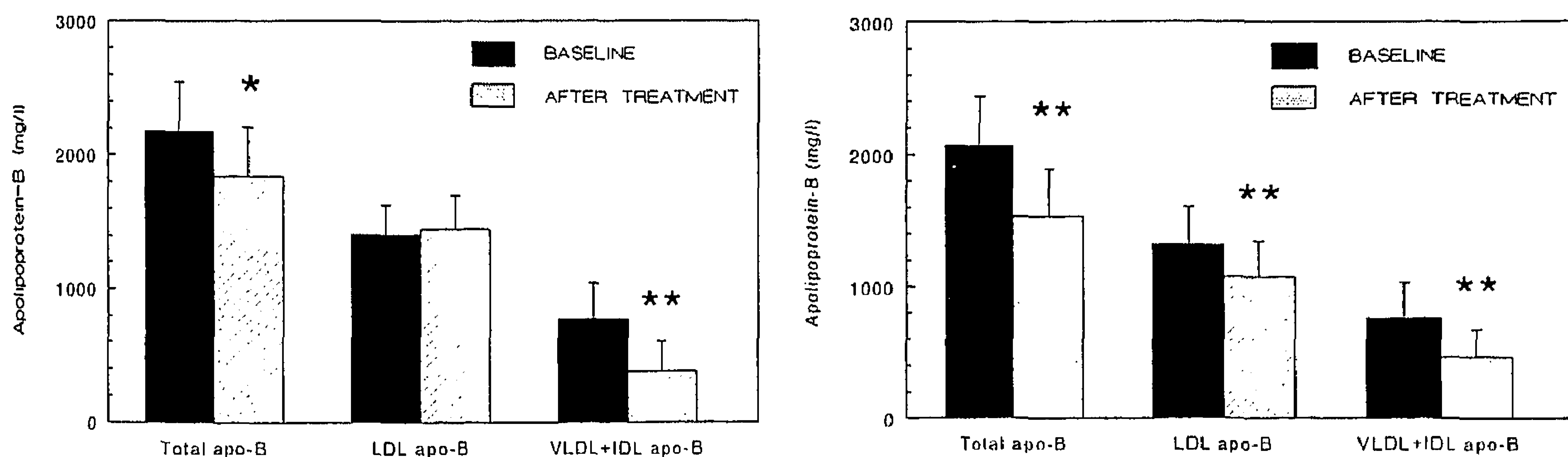


Fig. 2. Effect of treatment with either gemfibrozil ($n = 40$, left) or simvastatin ($n = 41$, right) on apolipoprotein-B-containing lipoproteins in total plasma, the LDL fraction and the VLDL + IDL fraction of patients with familial combined hyperlipidaemia, before (solid columns) and after (hatched columns) treatment. * $P = 0.001$, ** $P = 0.0001$.

Table 3

Lipoprotein (a) [Lp(a)], apolipoprotein-AI (apo-AI) and apolipoprotein-CIII (apo-CIII) at the end of the placebo period (= before) and after treatment (= after) with either gemfibrozil ($n = 40$) or simvastatin ($n = 41$) of patients with familial combined hyperlipidaemia

	Drug	Before	After	Delta (%)	P
Lp(a)	G	18.0	20.7	10.2 ± 38.9	n.s.
	S	19.6	26.1	14.9 ± 31.1	0.015
Apo-A I	G	131.5 ± 24.7	134.4 ± 24.1	2.9 ± 12.2	NS
	S	136.7 ± 25.4	133.7 ± 22.2	-0.1 ± 18.1	NS
Apo-CIII	G	14.8 ± 3.4	10.6 ± 2.3	-26.0 ± 17.2	< 0.0001
	S	14.7 ± 4.7	13.6 ± 3.6	-6.4 ± 20.3	$^a 0.005$

Values are presented in mg/dl as median for Lp(a) and as mean \pm SD for apo-AI and apo-CIII. S = simvastatin; G = gemfibrozil; n.s. = not significant. ^a $P < 0.0001$, S versus G.

and simvastatin ($r = 0.69$ and $r = 0.67$; $P = 0.001$, respectively).

3.5. Effect of treatment on Lp(a)

Before and after therapy Lp(a) values showed a wide distribution. Effects on median levels are summarized in Table 3. The effect of gemfibrozil was not significant, 18 out of 40 patients showed a decrease, but conversely, 27 out of 41 patients showed an increase in Lp(a) levels on simvastatin.

4. Discussion

In spite of often mildly elevated lipid levels compared to other lipid disorders, there is a high prevalence of cardiovascular diseases in FCH families. Consequently, family screening and pharmacological intervention in affected individuals may be indicated [21]. The increased tendency to premature cardiovascular disease in these patients must be related to the increased pool of triglyceride-rich apo-B-containing lipoproteins in conjunction with elevated LDL-cholesterol levels [22,23]. In addition, hypertriglyceridaemia, as found in FCH with type IIb and IV phenotypes, is associated with augmented exchange of lipids between VLDL, HDL and LDL, mediated by cholesteryl transfer protein (CETP), which may result in more small dense atherogenic LDL particles in total LDL [24,25]. Elevated apo-CIII concentra-

tions may be associated with hypertriglyceridaemia, because of a possible inhibition of LpL activity by apo-CIII [26]. Furthermore, decreased HDL-cholesterol, apo-AI concentrations and elevated concentrations of Lp(a) may also be involved in the atherogenesis in FCH [13,27].

Recently, large prevention trials and studies with solid cardiovascular end-points have now established that cholesterol-lowering results in a decrease in mortality from cardiovascular disease, and regression of atherosclerotic lesions [28–31]. Although the study populations did not meet the FCH diagnostic criteria, it can be deduced from these results that lipid-lowering may affect mortality and morbidity from cardiovascular events in FCH patients as well, because lipid parameters associated with an increase in cardiovascular risk were also influenced in the present study.

The observed reduction of total plasma cholesterol and plasma triglyceride concentrations with simvastatin (-22.4 and -9.5% , respectively) and gemfibrozil (-14.6 and -45.5% , respectively) after 12 weeks of treatment in our study are in accordance with previous reports [32,33]. To investigate the effect of both therapies on triglyceride-rich apo-B-containing lipoproteins, VLDL and IDL particles were isolated together. This explains the relatively large contribution of VLDL + IDL-cholesterol and the relatively small contribution of LDL-cholesterol to total plasma cholesterol, both before and after treatment. The increase in HDL-cholesterol with gemfibrozil therapy agrees with previous studies as well [32,34], but the increase in HDL on simvastatin, despite a less pronounced reduction of triglycerides, is larger than previously reported [32]. The mean apo-AI concentration was unaffected, despite an increase in HDL-cholesterol and may reflect HDL-cholesterol particles containing less protein [35]. The effects on LDL-cholesterol are comparable with other reports [32,33]. The depletion of triglycerides in the VLDL + IDL fraction, leading to more small dense particles, which are more likely to be converted into LDL particles, is suggested to be a cause of the observed increase in LDL-cholesterol after gemfibrozil [36]. On the other hand, a triglyceride reduction in the LDL particle, more pronounced after gemfibrozil, yields more buoyant LDL subfractions, as we have demonstrated before [5,37]. These buoy-

ant LDL subfractions may be less atherogenic because an increased resistance against oxidative modification has been described [18]. We observed that simvastatin most effectively reduced the total LDL-cholesterol concentration, but did not affect the LDL subfraction profile of FCH patients [5], which was recently demonstrated for pravastatin as well [38].

All patients had moderate to severe elevations of apo-B levels. Both therapies reduced total apo-B, but in accordance with the reduction of total apo-B-containing particles, simvastatin reduced apo-B in both the VLDL + IDL and LDL fraction more effectively than gemfibrozil, which only reduced VLDL + IDL-apo-B. Since VLDL + IDL and LDL particles contain only one molecule of apo-B per particle, a reduction of apo-B observed in these fractions reflects a reduction of the number of potential atherogenic particles per isolated fraction.

It has been suggested that an elevated apo-CIII concentration may be a metabolic marker for FCH. The decrease in the apo-CIII concentrations in this study, however, significantly correlated with the decrease in triglyceride-rich lipoproteins and paralleled the reduction of the particle numbers in the VLDL + IDL fraction. Recently, Patsch et al. found a lower apo-CII/apo-CIII ratio, due to an elevated apo-CIII concentration, in VLDL particles of patients with type IIb hyperlipidaemia, which was related to sequence variations in the minor allele of the AI/CIII/AIV gene cluster on chromosome 11 [39]. From our results, it remains to be seen whether increased apo-CIII concentrations have some role in the pathophysiology of FCH and can serve as a metabolic marker.

So far, the effects of hypolipidaemic drugs on Lp(a) levels have not been very successful. Studies with simvastatin demonstrated either no effect [40] or an increase in Lp(a) concentrations [15]. Although it has been shown that Lp(a) may be bound to triglyceride-rich lipoproteins [41] and that LpL-mediated clearance of triglyceride-rich lipoproteins influences Lp(a) concentrations [14], the mechanism behind this association remains unclear. Synthesis of Lp(a) in the liver may share some characteristics with VLDL synthesis, which is dependent on the amount of free fatty acids to the liver cell [42]. It is still unclear which causes underlie this possible increased supply of free fatty acids to the liver cell in

FCH. Gemfibrozil, however, which may have some effect on the release of fatty acids from adipose tissue, had no significant effect on Lp(a) concentrations.

In conclusion, we directly compared the effects of two drugs with different working mechanisms in a well-defined large group of FCH patients in order to support the choice of treatment for this heterogeneous lipid disorder. None of these agents completely normalized the lipid and lipoprotein profiles. However, each agent has its specific effect in the treatment of subjects with both elevated total plasma cholesterol and triglyceride concentrations. The use of drugs should be based on which lipoprotein fraction is elevated the most during several visits to the out-patient clinic, and probably a combination of gemfibrozil and simvastatin may be the therapy of choice in selected FCH patients with a high risk of cardiovascular disease.

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